# SYNOPSIS

# SYNTHESIS AND CHARACTERIZATION OF NEW SUBSTITUTED HETEROCYCLIC COMPOUNDS (OXOINDOLINES, QUINOXALINE 1,4-DI-N-OXIDES, 1,2,4-TRIAZOLES, CHROMANOISOXAZOLE DERIVATIVES) AND STUDY OF THEIR ANTIMICROBIAL, ANTICANCER, ANTI-INFLAMMATORY ACTIVITIES AND MOLECULAR DOCKING (RESPECTIVELY)

*SYNOPSIS OF THESIS SUBMITTED FOR THE AWARD OF THE DEGREE OF*

**DOCTOR OF PHILOSOPHY**

**IN CHEMISTRY**

***Submitted by***

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# SYNOPSIS

The thesis comprises of five chapters. Chapter-1 deals with an overview of heterocyclic compounds and their biological significance and pharmacological activity besides other applications of oxoindolines, quinoxalines, isoxazoles, and 1, 2, 4-triazoles.

Chapter-2 describes Synthesis, characterization of 4(2-(2-oxoindoline-3-ylidene) amino cyclo pent-1-ene-carbothioyl)thio derivatives and evaluation of their antimicrobial activities.

Chapter-3 deals with the Synthesis and characterization of 5,6-substituted,2,3-diaryl Quinoxaline 1,4-di N-Oxide derivatives & 7-substituted-2,3-diphenylpyrido[2,3-b] pyrazine1,4-di-N-oxides and Investigations of their Anticancer activity besides Microbial activity.

Chapter-4 describes the Synthesis and characterization of 3-substituted phenyl-5-(2",2"-dimethyl,7"-hydroxychroman)isoxazoles & 3-(4'-chlorophenyl)-5-(3", 4", 9", 10"-tetrahydro-2",2",8",8"tetramethyl-2"H,8"H-dipyranylbenzo[1,2-b:3,4-b']) isoxazole and study of their biological activity & Molecular Docking studies.

Chapter-5 describes the Synthesis and characterization of 5-((3-(4-substituted phenyl)-5-mercapto-4H-1, 2, 4-triazol-4-yl)diazenyl)naphthalene-2-ol derivatives and study of their anti-inflammatory activity besides microbial activity.

## Chapter-I: An overview of heterocyclic compounds and their biological significance.

Heterocyclic compounds are widely distributed in nature and important activities are associated with this class of substances. The paramount importance of heterocyclic compounds in natural product chemistry and pharmacology constantly drive the search for new methods for the construction of heterocyclic unit viz. Oxoindolines, Quinoxalines, Isoxazoles and Triazoles. The chemistry of the above is briefly described in sections A,B,C,&D respectively.

### Sec-A: Chemistry of OxoIndolines:

Oxoindolines are aromatic heterocyclic compounds, which possess variety of biological activities viz., antimicrobial, antiviral, antifungal, anti-inflammatory, analgesic activity etc. Some of oxoindolines are used as new apoptosis inducers which play a crucial role in normal cell development and tissue homeostasis. Apoptosis is used by organism to control their cell numbers and to eliminate unneeded or damaged cells. A large number of oxoindoline derivatives have been incorporated into a wide variety of chemotherapeutical agents and is correlated to their apoptosis inducing ability (as apoptosis inducers or as potential anticancer agents).

**Sec-B**: **Chemistry of Quinoxalines:**

A quinoxaline, also called a benzopyrazine, in organic chemistry is a heterocyclic compound containing a ring complex made up of benzene ring and a pyrazine ring. It is isomeric with other naphthyridines including quinazoline, phthalazine and cinnoline. Quinoxalines are used as dyes, pharmaceuticals and antibiotics such as echinomycin, levomycin and actinoleutin. Studies were carried out in order to explore the antitumoral properties of quinoxaline compounds. Recently, quinoxalines and its analogs have been investigated as "catalysts". The wide spread activity of quinoxaline 1,4 di-N-oxides (QdNO's) can be associated with generation of free radicals. QdNO's were first prepared as potential antagonists of vitamin K activity, but the studies are to be extended. They have been reported for their application in dyes, efficient electroluminicent materials, and organic semiconductors.

**Sec-C: Chemistry of Isoxazoles:**

Isoxazoles possess interesting medicinal properties and have industrial applications. Many biologically active isoxazoles and reduced isoxazole derivatives have been reported to be useful in psychotherapy and isoxazole-steroids show anabolic activity. Isoxazole derivatives were used as inhibitors for ulcers, lipoxygenase and acetyl choline esterase. They exhibit a variety of pharmacological activities like hypoglycemic, analgesic, antiarrythymic, antitumor etc., spiro isoxazolines and benzofuroisoxazoles were used as anticonvulsants. Isoxazolyl napthoquinones act as potential trypanocidal and antibacterial agents. Heterolyptic

tetraorganotins have insecticidal properties. Some new dyestuff's containing isoxazole moieties were found to give excellent results when applied on wool, polyester and their blend. Some novel isoxazoles, like Broxaterol, show marked bronchodialating activity. Some Isoxazole derivatives are used in liquid crystalline mixtures, which are useful for display devices.

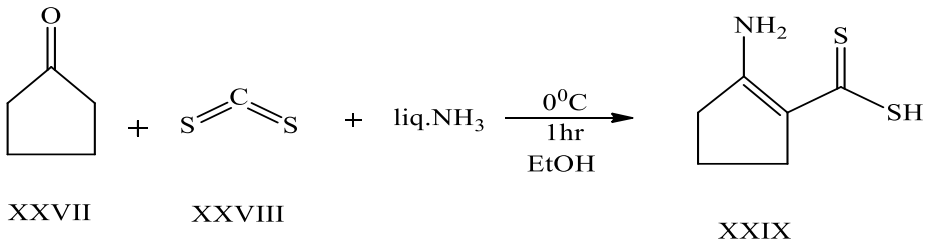
**Sec-D: Chemistry of 1,2,4-triazoles:**

The chemistry of 1, 2,4-triazoles and their fused heterocyclic derivatives has received considerable attention, owing to their synthetic and effective biological importance. A large number of 1,2,4-triazole containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial agents and atimycotic activity such as fluconazole, intraconazole, voriconazole. Also there are known drugs containing the 1,2,4-triazole group eg., Triazolam, Alprazolam, Etizolam, and Furacylin. Moreover, sulphur containing heterocycles represent an important group of sulphur compounds that are promising for use in practical applications. Among these heterocycles, the mercapto- and thione-substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives, such as antibacterial, antifungal, antitubercular, antimycobacterial, anticancer, diuretic and hypoglycemic properties. Triazole derivatives are showing very promising and excellent therapeutic effectiveness. The major activities exhibited by these derivatives

include insecticidal, antifungal, antiviral, antibacterial, sedative, hypnotic, anticonvulsant and anti-inflammatory action. These heterocyclics are emerging as the most explored search to obtain clinically significant compounds. The highly explored isomers of triazole being the 1,2,4-triazoles.

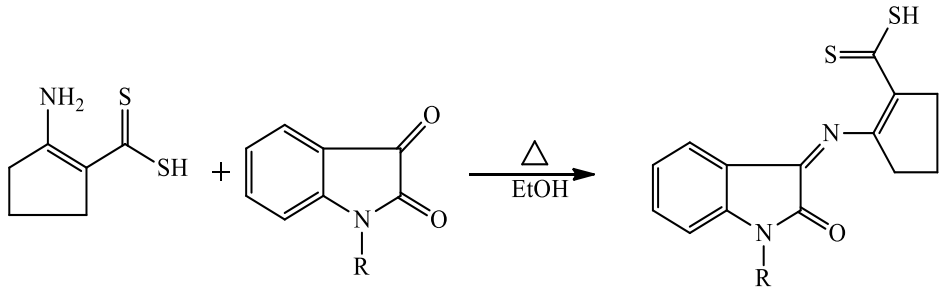
**Chapter-II: synthesis, characterization of 4(2-(2-oxoindoline-3-ylidene) amino cyclo pent-1-ene-carbothioyl) thio derivatives and evaluation of antimicrobial activities**.

Isatin (Indole 2,3-dione) is an endogenous compound, which were synthetically versatile substrates, useful for the synthesis of large variety of heterocyclic compounds. The synthetic versatility of Isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives. Prompted by the biological properties of oxoindoline derivatives, it was decided to synthesize various oxoindoline derivatives of isatin with aryl substrates and to screen for their antimicrobial activities. The oxoindoline derivatives were synthesized by condensing 2-amino cyclo pent 1-ene carbodithioic acid (prepared from cyclopentanone & carbon disulphide) with Isatin. All the synthesized compounds were screened for their Antibacterial and Antifungal activities, which exhibited promising results. **Scheme-4, 5 & 6** illustrate the synthetic methodology for the preparation of novel (Z)-4-substituted-((2-((2-oxoindolin-3-ylidene) aminocyclopent-1-ene carbothio-yl)thio derivatives.(XXXIIa-XXXIIe). All the synthesized compounds were screened for their Antibacterial and Antifungal activities, and the results are presented.



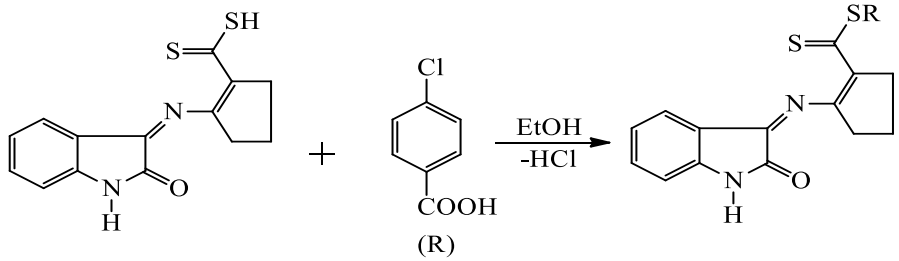

XXVII XXVIII XXIX

Scheme 4

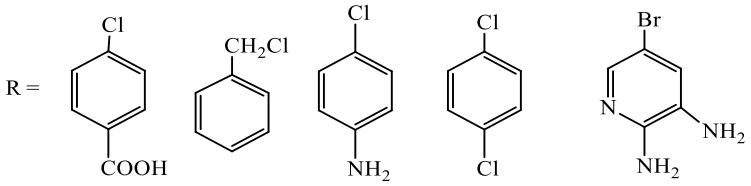



XXIX XXX XXXI

Scheme 5




XXXI XXXII(a-e)




**XXXIIa** **XXXIIb** **XXXIIc** **XXXIId** **XXXIIe**

Scheme 6

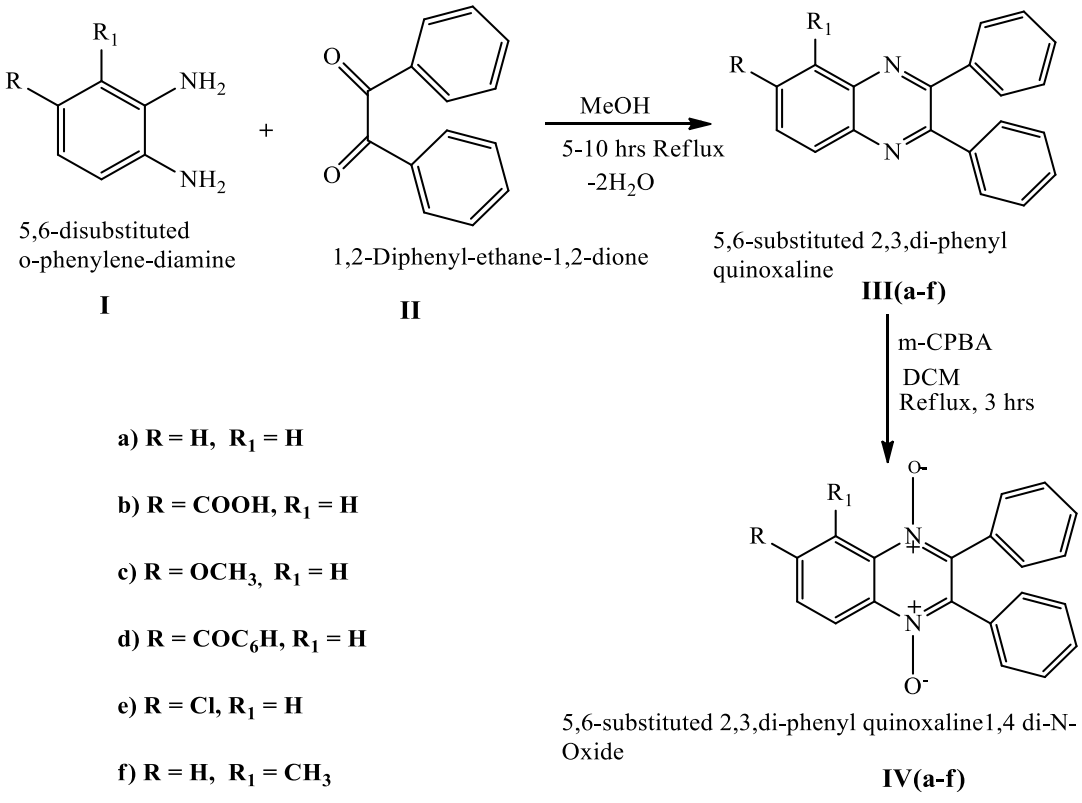
**Study of Antimicrobial Activity:**

The synthesized compounds (XXXIIa-XXXIIe) were evaluated *in vitro* for antifungal activity by using agar well diffusion method. The test organisms are *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170). They were cultured on potato dextrose agar medium. Fluconazole was used as a standard reference and DMSO was used as a solvent. The compounds XXXIIa, XXXIIb, XXXIIc, exhibited potent activity against *S.cerevisiae.*

The synthesized compounds (XXXIIa-XXXIIe) have also been tested for antibacterial property against gram positive bacteria *S.aureus* (MTCC 3160), *B.subtilis* (MTCC 441) and *B.cereus* (MTCC 430); gram negative bacteria *P.aeruginosia* (MTCC 424), *E.coli* (MTCC 443) and *P.vulgaris*. The compound viz; XXXIIa, XXXIIc, XXXIIe showed potent activity (nearly equal to the inhinition zone value of streptomycin) against *P.vulgaris*, *S.aureus*, *E.col*i, and *P.aeruginosa*. The results were compared employing streptomycin as standard reference drug. Compounds with carboxylic and amine substituents showed better activity than other substituents.

**Chapter-III: Synthesis and Characterization of 5,6-substituted,2,3-diaryl Quinoxaline 1,4-di N-Oxide derivatives & 7-substituted-2,3-diphenylpyrido[2,3-b] pyrazine1,4-di-N-oxides and Investigations of their Anticancer activity besides Microbial activity.**

Quinoxalines are versatile class of nitrogen containing heterocyclic compounds and they constitute useful intermediates in organic synthesis and also widely used in dyes, pharmaceuticals. Quinoxalines and their derivatives are found to be associated with various biological activities. Oxidation of both nitrogens of the quinoxaline ring dramatically increases the diversity of certain biological properties. In continuation of our studies on quinoxaline compounds, substituted quinoxaline 1,4 di-N-Oxides containing electron donating and electron withdrawing groups were synthesized and screened for Anticancer activity and Antimicrobial activity. **Scheme-14&15** illustrate the synthetic procedure for the preparation of substituted quinoxaline 1, 4-di N-Oxides (IVa-IVf) & 7-substituted-2,3-diphenylpyrido[2,3-b] pyrazine1,4-di-N-oxides (VIIIa-VIIIb).




5,6-disubstituted o-phenylene-diamine

**I**

1,2-Diphenyl-ethane-1,2-dione

**II**

5,6-substituted 2,3,di-phenyl quinoxaline

**III(a-f)**

**a) R = H, $R_1$ = H**

**b) R = COOH, $R_1$ = H**

**c) R = $OCH_3$, $R_1$ = H**

**d) R = $COC_6H$, $R_1$ = H**

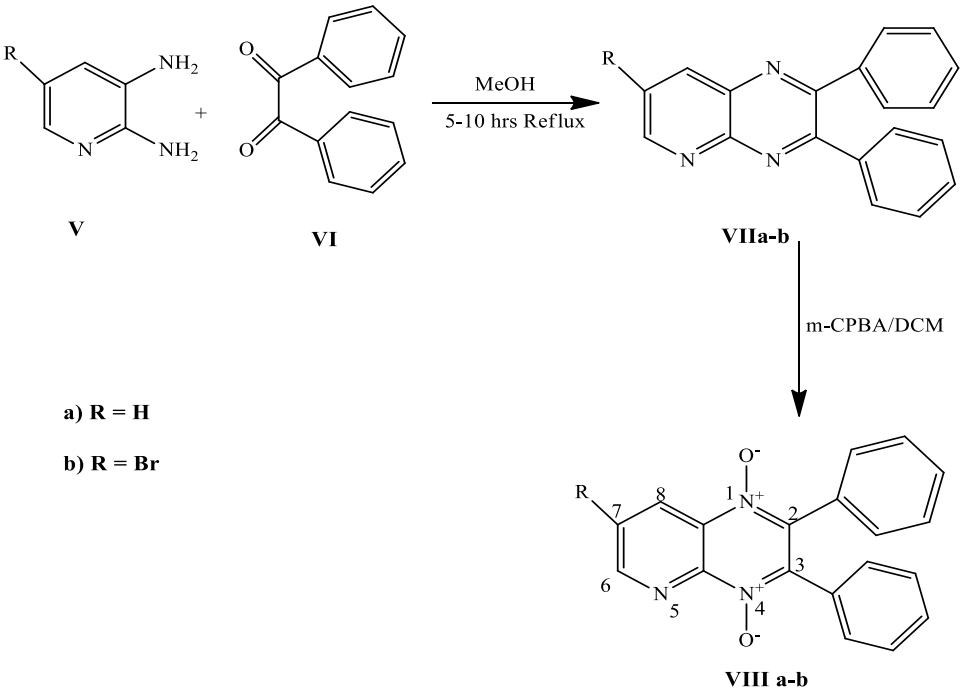
**e) R = Cl, $R_1$ = H**

**f) R = H, $R_1$ = $CH_3$**

5,6-substituted 2,3,di-phenyl quinoxaline1,4 di-N-Oxide

**IV(a-f)**

**Scheme 14**




**V**

**VI**

**VIIa-b**

**a) R = H**

**b) R = Br**

**VIII a-b**

**Scheme 15**

**Cytotoxic Studies**:

Cytotoxic investigation were carried out at M/s Natco Research Pharma, (NRC), Sanatnagar, Hyderabad, in collaboration with Dr.A.K.S.Bhujanga Rao, President (R&D) NRC, Hyderabad and Dr.Suneel, NRC, Hyderabad.

The *in vitro* cytotoxicity studies of synthesized compounds IVa, IVc, IVd, IVe, & VIIIb were performed on four different cell lines and out of these, three are lung cancer cell line (A549, NC1H292, and HCC827) and other is renal cancer cell line (786O respectively) and the cell viability was measured. The activities of the compounds were compared to that of reference standard drug Botrezomib. The compounds IVa, IVc, IVd showed potent $IC_{50}$ values against HCC827 and NC1H292 cell lines, when compared with the standard. For 7860, A549 cell lines the activity is moderate. It was observed from the results that the synthesized compounds IVa, IVc, IVe, exhibited significant anticancer activity on a lung cancer cell lines viz; HCC827 and NC1H292.

**Antibacterial Activity:**

The antibacterial activities of the synthesized compounds IVa, IVb, IVc, IVd, IVf, VIIIb were determined by the agar well diffusion technique. All the tested compounds along with standard streptomycin was screened *in vitro* for antibacterial activity against gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 430), gram negative bacteria

*Peudomonas aeruginosa* (MTCC 424) and *Escherichia coli* (MTCC 443). The solutions of each tested compound were dissolved in dimethyl sulphoxide (DMSO). Compounds IVb, IVc, IVd exhibited moderate activity against *P.aeruginosa*. The compounds IVb, IVc, IVd and IVe showed high activity against *B.subtilis*, whereas IVa and VIIIb show moderate activity. The compounds IVd, IVe show high activity against *E.coli* and IVa. IVb, IVc. VIIIb show moderate activity. Compounds VIIIb, IVe exhibited high activity against *S.aureus*, IVa, IVb, IVc, IVd exhibited moderate activity. All the compounds showed lower (less significant) activity against *B.cereus*. All these compounds are compared with the standard reference (streptomycin) for their antibacterial activities.

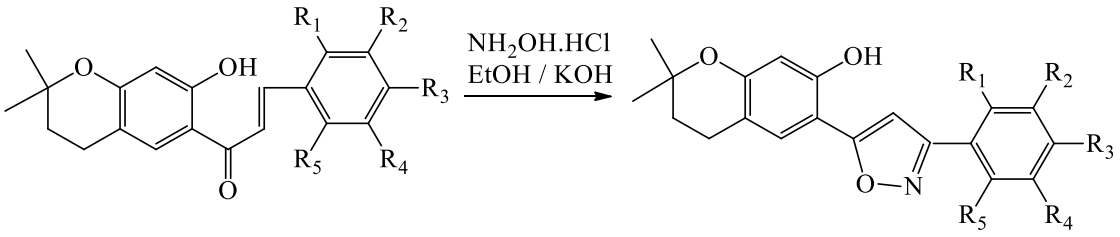
**Antifungal activity:**

All the synthesized compounds IVa, IVb, IVc, IVd, IVf and VIIIb were evaluated *in vitro* antifungal activity by using agar well diffusion method; the test organisms employed *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170). Nystatin was used as a standard reference and DMSO was used as solvent (control), which did not possess any inhibition zone. Compounds IVc, IVb, IVe, VIIIb showed moderate antifungal activity when compared with standard reference Nystatin.

**Chapter-IV: Synthesis and characterization of 3-substituted phenyl-5-(2",2"-dimethyl,7"-hydroxychroman)isoxazoles & 3-(4'-chlorophenyl)-5-(3",4",9",10"-tetrahydro-2",2",8",8"tetramethyl-2"H,8"H-dipyranylbenzo[1,2-b:3,4-b']) isoxazole and study of their biological activity & Molecular Docking studies:**

Isoxazole is π-excessive five membered ring. The naturally occurring antibiotic-cycloserine, isocarboxyzide, ibotenic acid and muscimol, are isoxazole derivatives. This chapter is divided into three sections; Section-A, which describes the synthesis and characterization of some new isoxazoles viz. 3-substituted phenyl-5-(2",2"-dimethyl,7"-hydroxychroman) isoxazoles [compounds 26-30]; in Section B, Synthesis, characterization of new chromanoisoxazole,3-(4'-chlorophenyl)-5-(3",4",9",10"-tetrahydro-2",2",8",8"tetramethyl-2"H,8"H-dipyranyl benzo[1,2-b:3,4-b']) isoxazole is given in Scheme-X & Scheme-XII and Section-C, gives an account of biological activity and molecular docking studies of chromanoisoxazoles.

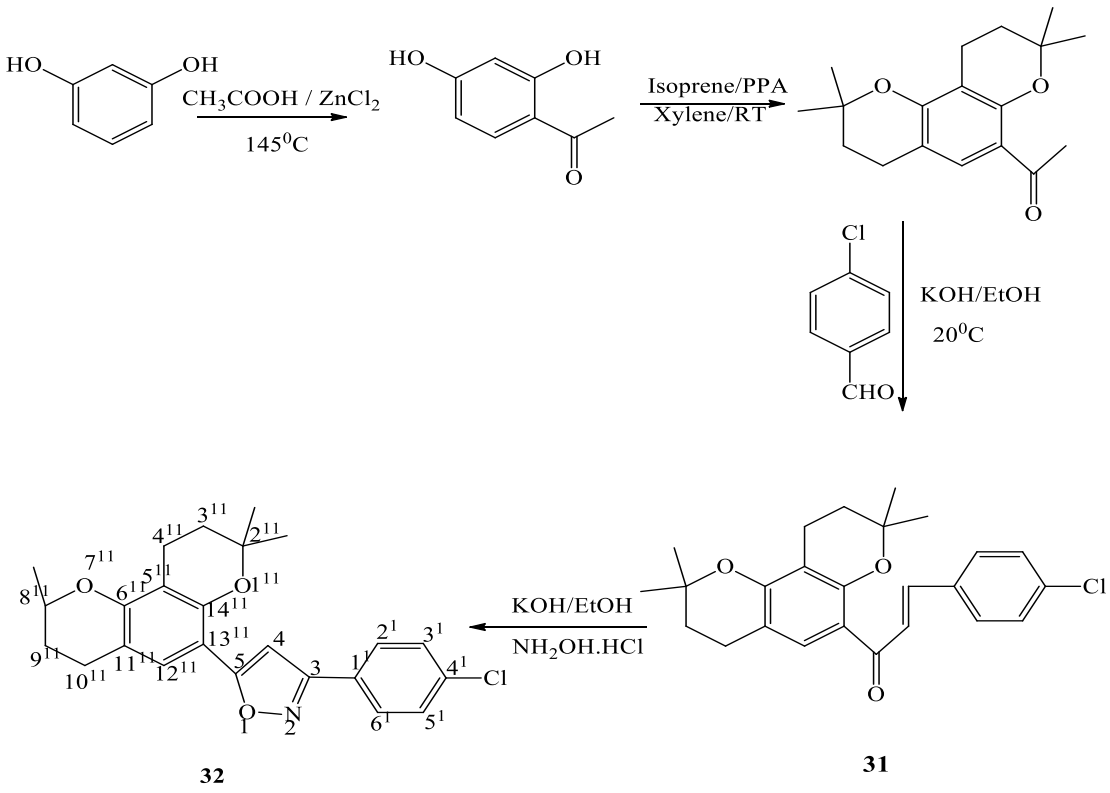
The method employed for the synthesis of isoxazoles has been the common (3+2) route. Chalcones form the $C_3$-frame work, and these chalcones, on condensation with hydroxylamine hydrochloride result the formation of 3-substituted phenyl, 5-(2",2"-dimethyl,&"-hydroxy chroman) isoxazoles. They were characterized using elemental analysis, IR, $^{1}$HNMR, $^{13}$CNMR, etc.




**21-25** **26-30**

**Scheme X**

**21. $R_1$, $R_4$, $R_5$ = H & $R_2$, $R_3$ = $OCH_3$**
**22. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = N $(CH_3)_2$.**
**23. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = Cl.**
**24. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = $OCH_3$.**
**25. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = CN.**
**26. $R_1$, $R_4$, $R_5$ = H & $R_2$, $R_3$ = $OCH_3$.**
**27. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = N $(CH_3)_2$.**
**28. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = Cl.**
**29. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = $OCH_3$**
**30. $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = CN.**



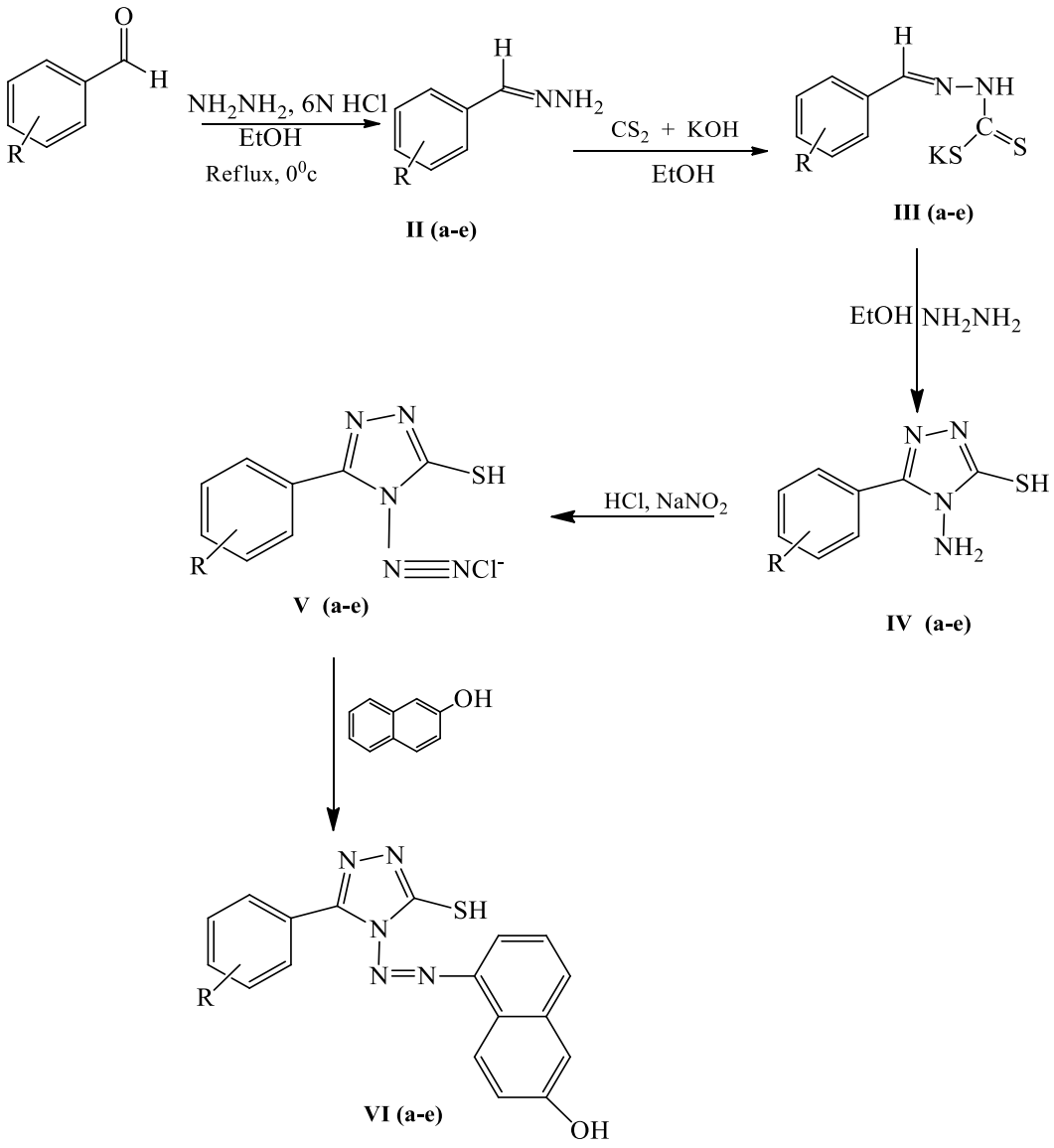

**32** **31**

**Scheme-XII**

The biological activity [viz. antimicrobial activity (employing agar well diffusion method)] and pharmacological activity studies of 3-substituted phenyl 5-(2",2"-dimethyl,&"-hydroxy chroman) isoxazoles synthesized, were reported in this chapter. Antibacterial activity was determined against, Gram-positive bacteria-*Bacillus subtilis & Bacillus pumilus* and Gram-negative bacteria, *Escherichia coli & Proteus vulgaris*, at concentrations of 5, 10, 20, 50, 100 and 200µg/ml (to find minimum inhibitory concentrations (MIC)). The antifungal activity of the compounds was tested against two fungi, *Rhizopus oryzae* and *Aspergellius niger*. The *in vitro* experimental findings are in line with the results of the Molecular Docking studies.

**Chapter-V: Synthesis and characterization of 5-((3-(4-substituted phenyl)-5-mercapto-4H-1, 2, 4-triazol-4-yl) diazenyl) naphthalene-2-ol derivatives and study of their anti-inflammatory activity besides microbial activity:**

The triazole nucleus is an important five membered nitrogen containing heterocycles, In the present chapter, we describe the synthesis, characterizations of substituted 1, 2, 4-triazoles in part-A and anti-inflammatory activity as well as antimicrobial activities of the synthesized 1, 2, 4-triazole derivatives **(VIa-VIe)** in part-B**. Scheme-I** illustrates the synthetic process for the preparation of 5-((3-(4-substituted phenyl)-5-mercapto-4H-1,2,4-triazol-4-yl)diazenyl)naphthalene-2-ol. **(VIa-VIe).**




**R = a) H,**
**b) Cl,**
**c) Br,**
**d)$NH_2$,**
**e)$NO_2$,**

**Scheme I**

**Anti-inflammatory activity of the synthesized 1,2,4-Triozole derivatives**

The synthesis of heterocyclic rings containing nitrogen atom and their importance in medicinal chemistry is noteworthy. Increasing attention has been paid over the past two decades to the chemistry of triazole derivatives. In continuation of our interest for examining the biological activity of the synthesized molecules, we carried out the *in vitro* anti-inflammatory activity of the synthesized molecules. Anti-inflammatory refers to the property of a substance that reduces inflammation. The *in vitro* anti-inflammatory activity of the synthesized triozole compounds were performed by using the 5-Lipoxygenase (5-LOX) Assay compared to that of the reference standard Curcumin. These activities were performed at M/s.Laila Neutraceuticals (R&D Centre), Vijayawada, in association with Dr.A.Trimurthulu, President (R&D), Laila Neutraceuticals, Vijayawada.

The lipoxygenase inhibitory concentrations were expressed as $IC_{50}$ (µg/ml). The compound VIb and VIe exhibited more potent activity with $IC_{50}$ 6.98 µg/ml and 8.0µg/ml. and are first time reported. Compound VIc exhibited $IC_{50}$ value 20.84µg/ml which can also be categorized as potent compound. The remaining compounds VIa and VId exhibit $IC_{50}$ more than 100µg/ml. "Curcumin" the reference standard, exhibits the $IC_{50}$ value 12.79µg/ml under similar test conditions. The results are presented in fig 19 and 20. Hence it is concluded that compounds VIb and VIe possess notable anti-inflammatory activity, when compared with the reference

standard. The compound VIc also exhibits anti-inflammatory activity compared with reference standard "Curcumin**.**

**Antibacterial activity**

The antibacterial activities of the synthesized compounds were evaluated *in vitro* at different concentrations (25µg, 50µg, 100µg and 200µg). The compounds were tested against gram positive bacteria: *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 430), and gram negative bacteria: *Pseudomonas aeruginosa* (MTCC 424) *Escherichia coli* (MTCC 443) and *Proteus vulgaris*. The inoculated sterilized nutrient agar media was poured into petri dishes and allowed to solidify. 6mm wells were made on the agar surface, into each of these wells, 30µl of the test compound with different concentrations /reference standard/control was added by using a micropipette. Streptomycin was used as standard reference and DMSO was used as a control (solvent) which did not possess any inhibition zone. The plates were incubated at 37ºC for 24 hours for bacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in triplicate and the average values were tabulated.

**Antifungal activity**

All the synthesized compounds were evaluated, *in vitro* for antifungal activity, by using agar well diffusion method. The test organisms are *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170) were used. They were grown on

potato dextrose agar medium. The plates were incubated at 28°C for 24 hrs and the zone of inhibition was measured in mm. Fluconazole was used as a standard reference and DMSO was used as a solvent (control), which did not possess any inhibition zone.

The screening result indicate that three compounds exhibited potent anti-inflammatory and antimicrobial activities, due to the presence of triazole ring system in synthesized compounds. It can be noted that compounds Cl, Br, $NH_2$, and $NO_2$ as substituents in 4th position **(VIa-VIe)** showed the maximum inhibitory effect against one or more type of bacteria. Among the synthesized compounds, compound **VIe** showed worthy antibacterial activity and **VIa** showed moderate antibacterial activity. From antifungal activity, it is clear that, compounds **VIb, VIc** showed mild activity and compound **VId** showed moderate antifungal activity. From the results, we can observe that the compound showed lower fungicidal effect compared with their bactericidal effect. In *in vitro* anti-inflammatory activity, among the selected synthesized compounds, compound **VIb, VIc, VIe** showed potential activity compared to reference standard.